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p53 Function and Dysfunction

Minireview

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The word "cancer" is used to describe a group of heterogeneous pathologic states in which cells multiply abnormally and invade surrounding tissues. There are hundreds of different kinds of cancers, at least one originating from nearly every cell type in the mammalian organism. One long-standing hope has been that the same biochemical pathway for controlling growth is disrupted in many different kinds of cancers, despite their biologic heterogeneity; this would provide a common denominator for understanding, treating, and preventing these diseases. The pathway involving p53 fulfills this hope, as alterations of this tumor suppressor gene appear to be involved, directly or indirectly, in the majority of human malignancies. This has in turn stimulated an intense search for the biochemical functions of p53 and the effects of mutation on these properties.

Biochemical Activities of the Wild-Type p53 Protein

Two lines of p53 investigation have converged in the past year. First, it was noted that p53 contained an acidic domain near its N-terminus that was similar to those previously noted in well-characterized transcription factors (Fields and Jang, 1990; Raycroft et al., 1990). When this acidic domain was fused to the DNA-binding region of GAL4, the resulting chimeric protein could activate transcription from a *GAL4* operon. The activation domain has been recently mapped to the region lying between codons 20 and 42 (Unger et al., 1992; Miller et al., 1992) (Figure 1). Although many proteins contain such acidic regions, the strength of the activation and the nuclear localization of p53 suggest that p53 is involved in transcriptional control, either directly or through a complex with other proteins that bind to specific genes.

The second line of investigation illuminates this latter point. Through the testing of a large number of human genomic DNA clones, several have been identified that can bind to p53 in vitro (Kern et al., 1991; El-Deiry et al., 1992). Mapping of the p53-binding sites within these clones reveals that each contains two copies of the 10 bp motif 5′-PuPuPuC(A/T)(A/T)GPyPyPy-3′. One copy of the 10 bp motif is insufficient for binding, but binding is preserved when the two copies are separated by up to 13 bp of random sequence. The p53-binding sites have an obvious symmetry—four copies of the half-site 5′-(A/T)GPyPyPy-3′ are oriented in opposing directions. This suggests that p53 may bind to these sites as a tetrameric protein, which is consistent with biophysical studies indicating that p53 exists as a tetramer in solution (Stenger et al., 1992).

Two additional studies have confirmed that p53 can specifically bind to such sequences. From a large pool of random oligonucleotides, a small subset is selectable by virtue of binding to p53 (Funk et al., 1992). These synthetic oligomers share a 20 bp sequence very similar to the p53-binding sites described above. Anti-p53 antibodies and unidentified proteins from nuclear extracts appear to stabilize the complex of p53 with its binding sites (Funk et al., 1992; El-Deiry et al., 1992). The SV40 genome also contains a weak binding site for p53, which matches the 20 bp p53-binding site at 16 positions (Bargonetti et al., 1991).

If p53 binds DNA specifically and contains an acidic activation domain at its N-terminus, one would expect that p53 could activate the expression of genes adjacent to a p53-binding site. This expectation has been confirmed: cotransfection of a p53 expression vector with a plasmid containing a p53-binding site upstream of a reporter gene results in a high level of reporter activation in mammalian cells (Kern et al., 1992; Funk et al., 1992). This activation could have been an indirect one, perhaps in response to the numerous changes in gene expression and growth parameters induced by artificially high levels of wild-type p53. Indeed, p53 has been shown to affect the expression of several genes, few of which are likely to contain p53-binding sites (Ginsberg et al., 1991; Weintraub et al., 1991). Several additional experiments, however, strongly argue that p53 can directly activate transcription from p53-binding sites. First, the level of transcriptional activation precisely correlates with the strength of binding to p53-binding sites in vitro (Kern et al., 1992). Second, p53-binding site–mediated transactivation of reporter genes by p53 can be observed in yeast as well as in mammalian cells (Scharer and Iggo, 1992; Kern et al., 1992). If such effects are indirectly mediated through the induction of another gene product that actually binds to p53-binding sites in vivo, then the induction and function of this second gene product would have to be remarkably conserved in evolution, even though there is no known p53 homolog in

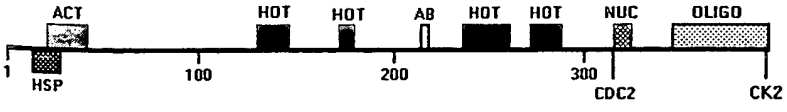

Figure 1. p53 Domain Map

HSP, a domain (amino acids 13–29) implicated in binding of heat shock proteins to mutant p53 (Lam and Calderwood, 1992); ACT, domain (amino acids 20–42) that, when fused to the DNA-binding domain of GAL4, activates transcription of genes downstream from GAL4-binding sites (Fields and Jang, 1990; Raycroft et al., 1990; Unger et al., 1992); HOT, "hotspots" (amino acids 129–146, 171–179, 234–260, 270–287) corresponding to evolutionarily conserved domains containing the most frequent sites of missense mutation (Hollstein et al., 1991); AB, region (amino acids 213–217) binding to mAb240 in some p53 mutants (Stephen and Lane, 1992); NUC, major nuclear localization signal (amino acids 316–325; Shaulsky et al., 1990); CDC2, serine-315 phosphorylated by $p34^{cdc2}$ kinase (Bischoff et al., 1990); CK2, serine-392 phosphorylated by casein kinase 2 (Fakharzadeh et al., 1991); OLIGO, domain (amino acids 344–393) required for p53 oligomerization (Milner and Medcalf, 1991). Human p53 contains 393 amino acids.

yeast. Third, transcriptional activation dependent on p53-binding sites can be demonstrated in an in vitro system using nuclear extracts and purified p53 (Farmer et al., 1992).

The Effect of p53 Mutations

The studies described above suggest that transcriptional activation through p53-binding sites is an important and biochemically assessable feature of the normal p53 protein. If this feature were central to the ability of p53 to suppress neoplastic growth, one might expect it to be disrupted by all p53 gene mutations. A large number of human p53 mutants have been described, the majority occurring as missense changes in one of the four "hotspots" shown in Figure 1. Representative mutants from each of these four regions have been tested for binding to p53-binding sites in vitro and for activation of p53-binding site reporter gene expression in vivo and in vitro. All mutants lose the ability to bind p53-binding sites and accordingly cannot activate the expression of adjacent reporter genes (El-Deiry et al., 1992; Kern et al., 1992; Scharer and Iggo, 1992; Farmer et al., 1992).

In addition to uniformly losing the ability to bind p53-binding sites, some mutants seem to change the global conformation of p53. For example, some missense mutants of p53 expose an epitope centered at codons 213–217, allowing it to react with monoclonal antibody 240 (Stephen and Lane, 1992). Similarly, some mutants of p53 allow binding to heat shock proteins (Sturzbecher et al., 1987), and others alter the acidic activation domain so that it cannot function when fused to the DNA-binding domain of GAL4 (Fields and Jang, 1990; Raycroft et al., 1990; Unger et al., 1992). Because these properties are found in only a fraction of p53 mutants, they are unlikely to be central to p53 function. However, the observations suggest that subtle mutations of p53 can affect the conformation of the entire protein, altering the structure of domains far removed from the sites of mutation (see Figure 1).

Such changes in conformation appear not only to affect p53 mutant molecules, but also can affect wild-type molecules complexed with the mutant proteins within the tetramer. Thus, cotranslation of mutant and wild-type proteins results in a change in conformation of the wild-type protein that mimics that of the mutant (Milner and Medcalf, 1991). Complexes of wild-type and mutant p53 protein cannot bind p53-binding sites in vitro or transcriptionally activate p53-binding site reporter genes in vitro or in vivo (Kern et al., 1992; Farmer et al., 1992). The binding to and consequent inactivation of wild-type p53 could explain the fact that some mutants of p53 can neoplastically transform cells, presumably by inhibiting endogenous wild-type p53 function in a dominant negative fashion. However, these dominant negative effects are not shared by all p53 mutants. p53 mutations that result in truncations will not exhibit dominant negative effects, because oligomerization is dependent on the presence of an intact C-terminus (Milner and Medcalf, 1991) (Figure 1). Moreover, some missense mutations exert more potent dominant negative effects than others and can be observed in vivo but not in vitro (Milner and Medcalf, 1991; Kern et al., 1992).

Genetic Alterations Affecting the p53 Pathway

In addition to intragenic mutations of p53, alterations of other genes apparently can lead to the same physiologic consequences. The first examples of this were provided by DNA tumor viral oncogenes, such as the large T antigen gene of SV40, the E1B gene of adenovirus, and the E6 gene of human papilloma virus. Each of these genes encodes proteins that bind to p53, and in the case of E6, this binding results in p53 degradation (Scheffner et al., 1990). Cells that express one of these viral oncoproteins and p53 cannot activate expression of p53-inducible reporter genes (Yew and Berk, 1992; J. A. Mietz and P. Howley, personal communication). The inhibition of p53-induced gene expression, and presumably of p53-mediated growth regulation, may be critical for virus replication and/or transformation.

The p53 pathway may also be disrupted by alteration of a cellular gene, *MDM2*. This gene was originally identified by virtue of its amplification in a spontaneously transformed mouse cell line (Fakharzadeh et al., 1991). The *MDM2* gene product has recently been shown to bind to p53 (Momand et al., 1992). As is the case with the viral oncoproteins, this binding appears to inhibit the ability of p53 to transactivate genes adjacent to p53-binding sites (Momand et al., 1992). This interference with p53 activity is not simply an experimental curiosity: the *MDM2* gene is amplified in a significant fraction of the most common human sarcomas, and the consequent overexpression of *MDM2* is likely to interfere with p53 activity (Oliner et al., 1992).

A Model for p53 Growth Control

The studies reviewed above suggest a model for p53 depicted in Figure 2. In this model, the wild-type p53 gene binds to p53-binding sites as a tetramer and stimulates the expression of downstream genes that negatively control growth and/or invasion. This expression can be lost in a variety of ways. In some tumors, a loss of one or both p53 alleles (through a large chromosomal defect or a localized deletion) reduces the concentration of p53 tetramers below that required to stimulate expression. In other tumors, a nonsense mutation results in the truncation of p53; the loss of the oligomerization domain at the C-terminus prevents the participation of the mutant p53 in tetramers. More commonly, one allele of p53 develops a missense mutation. This results in the reduction of tetramers composed totally of wild-type p53 monomers. Heterozygous mutant–wild-type tetramers do not function normally, and this dominant negative effect may be exacerbated by the increased stability (and therefore higher intracellular concentration) of the mutant protein compared with that of the wild-type protein.

A missense mutation of one p53 allele is often accompanied by a deletion of the other allele (usually through mitotic recombination), resulting in the absence of any wild-type p53 tetramers. This occurs in many tumors, including those of the colon, brain, lung, liver, and bladder (Hollstein et al., 1991). In cervical cancers, the expression of E6 results in the functional inactivation of p53 through binding and degradation. In soft-tissue sarcomas, the amplifica-

MECH.	CELLS	GENE	ALTERATION
--	NORMAL	----	NONE
1	OCCASIONAL TUMORS OF SEVERAL TYPES	p53	DELETION OF ONE (OR BOTH) ALLELES
2	FAIRLY COMMON IN LUNG, ESOPHAGUS, AND OTHER CANCERS	p53	NONSENSE OR SPLICE SITE MUTATION RESULTING IN TRUNCATION
3	COMMON IN COLON, BRAIN, LUNG, BREAST SKIN, BLADDER AND OTHER CANCERS	p53	MISSENSE MUTATION
4	COMMON IN CERVICAL CANCERS	E6	HPV INFECTION
5	COMMON IN SARCOMAS	MDM2	AMPLIFICATION

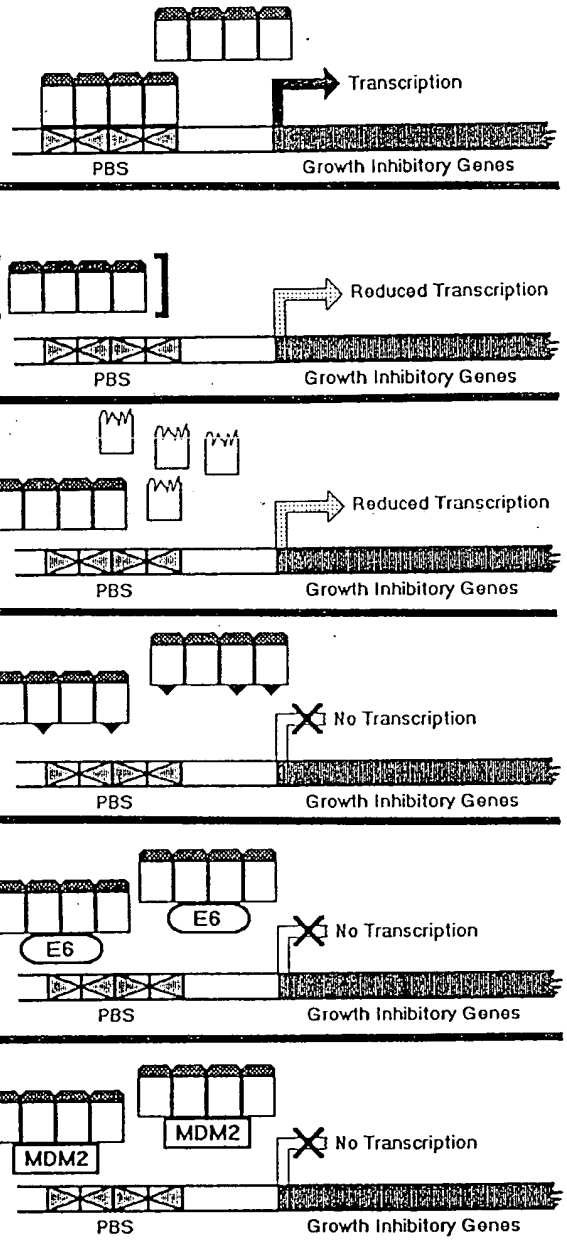

Figure 2. p53 Inactivation Mechanisms

p53 is postulated to bind as a tetramer to a p53-binding site (PBS) and activate the expression of adjacent genes that inhibit growth and/or invasion. Deletion of one or both p53 alleles reduces the expression of tetramers, resulting in decreased expression of these genes (mechanism 1). Mutations that truncate the protein do not allow oligomerization, thus resulting in a similar reduction of p53 tetramers (mechanism 2). Missense mutations resulting in dominant negative effects result in an even greater reduction of functionally active tetramers (mechanism 3). By binding to p53, the expression of E6 (mechanism 4) and increased expression of MDM2 (mechanism 5) result in functional inactivation of p53. It is not known whether E6–p53 and MDM2–p53 complexes inhibit binding to p53-binding sites, or whether they allow binding to p53-binding sites but inhibit transcriptional activation. E6 may also degrade p53 through ubiquitin-mediated proteolysis.

tion of *MDM2* and the binding of its product to p53 creates a similar loss of functional p53. In both cervical carcinomas with E6 expression and sarcomas with *MDM2* amplification, p53 mutations appear to be rare, whereas such mutations are common in other cervical cancers and sarcomas (Crook et al., 1992; Oliner et al., 1992). This is consistent with the expectation that only one mechanism for inactivating p53 in an individual tumor cell is required.

What Is Regulated by p53?

Although p53 can block the progression of the cell cycle when artificially expressed at high levels, it appears to play little role in normal cell cycle control. Thus, in mice

containing homozygous deletions and humans harboring germline mutations of p53, development is normal (e.g., Donehower et al., 1992; Malkin et al., 1990), and p53 protein is expressed at very low levels in most cell types. Emerging evidence, however, suggests that p53 may play an important growth-controlling role in stressed cells. In response to X-ray- or drug-induced damage, normal cells increase p53 expression and are arrested in the cell cycle until the damage is repaired. In contrast, cells with mutant p53 genes are only partially blocked, continue to divide, and then die (Kastan et al., 1991).

Developing tumor cells in situ may progress through a phase when they are stressed, perhaps as a result of anoxia or aneuploidy. As a result, wild-type p53 expression might be induced and limit growth, perhaps by stimulating p53-binding site–specific patterns of gene expression that inhibit cell cycle progression. Selection for mutant p53 genes at this juncture would allow further tumor expansion. This would explain why p53 mutations generally occur only late in tumor progression (Baker et al., 1990), when stress affords a selective advantage for cells containing p53 mutations; prior to this point, p53 is not expressed at significant levels and is not rate limiting for growth. It might also explain why tumor cells are often more sensitive to DNA-damaging agents such as those used in radiation and chemotherapy; this sensitivity may be a beneficial side effect of the loss of p53 function, which would otherwise limit cell death. p53 mutations may therefore constitute one of the few oncogenic alterations that increase rather than decrease the sensitivity of cells to antitumor agents.

Prospects for the Future

Among the most immediate issues is the identification of the genes adjacent to p53-binding sites that are regulated by p53 and that presumably control cellular growth. Although the data obtained so far have indicated that wild-type p53 can only directly affect gene expression through transcriptional activation, it is possible that p53 can also inactivate growth-promoting genes, just as other transcription factors can either activate or repress genes, depending on the sequence context of their binding sites. p53 is unlikely to interact with RNA polymerase directly; it will be important to discover the proteins that bridge p53 and this enzyme. In addition to MDM2, other proteins that negatively (or positively) affect p53 transcriptional control must exist, and it will be of interest to identify them. Alterations of the genes encoding these proteins could lead to a breakdown of the p53 pathway in the same way as *MDM2* in sarcomas. Finally, the three-dimensional structure of the p53 tetramer is likely to provide much important information. Particularly intriguing will be the elucidation of why the conformation of p53 and its functional properties are so easily disrupted by subtle mutations.

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